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False Tumor-Positive Lymph Nodes in Radioimmunodiagnosis and Radioimmunoguided Surgery: Etiologic Mechanisms

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We investigated the causes of false-positive (nontumor cell) focal uptake in radioimmunodiagnosis (RAID) and false-positive high counts in radioimmunoguided surgery (RIGS). Tissue blocks of two such RAID cases were recut and examined by immunohistochemistry (IH) (group 1). Lymph nodes in the drainage area of 14 colon cancers selected because of tumor-positive draining nodes were examined similarly (group 2). The lymph nodes in group 1 showed nontumor cell germinal center (GC) and rare macrophage (Mϕ) positivity with monoclonal antibody (mAb) CC49 to tumor antigen (Ag) TAG-72, the same Ag to which the mAb B72.3, used for the RAID studies, was directed. In group 2, CC49 staining was observed in the colon cancers, in noncellular tumor Ag in lymphatic channels, and in the GC of draining nodes in a pattern similar to that of follicular dendritic cells (FDC). An In-111-mAb/tumor Ag (TAG-72 or CEA) complex can result in false-positive RAID/RIGS studies by In-111 retained in the lysosomes of lymph node Mϕ, following attachment of the mAb to the Ag, and their catabolism in the Mϕ. An I-125-mAb to either tumor Ag could lead to false-positive RIGS studies due to its attachment to the Ag portion of ag/ab complexes affixed to the FDC in the GC of the lymph nodes draining a tumor.

KEY WORDS: **tumor antigen, TAG-72, CEA, follicular dendritic cells, immune complexes**

INTRODUCTION

In radioimmunodiagnosis (RAID), a patient is injected intravenously (i.v.) with a radiolabeled antitumor monoclonal antibody (mAb) and is then examined by scintigraphy hours to days later. The purpose is to detect tumors occult to other imaging methods. In radioimmunoguided surgery (RIGS) a patient has been injected with the same mAb, and when body background radioactivity has declined 3–4 weeks later, exploratory surgery is carried out. During surgery a handheld radiation detector is used by the surgeon to detect localized high counts, which is evidence for a tumor focus; this area is then excised or biopsied.

In the search for metastases from colorectal and ovarian cancer, false positivity of the draining lymph nodes has been encountered with both procedures [1–9]. Since this false positivity has occurred using mAb to antigens (Ags) shed by these tumors, TAG-72 [10] and carcinoembryonic antigen (CEA) [11], it has been attributed to tumor Ag in the lymph nodes [6–9]. We agree. However, the mechanisms are complex [12,13]. The rationale is that shed tumor Ag passes to the draining lymph nodes, where it undergoes catabolism but also induces Ab formation, with the subsequent formation of immune complexes and their deposition on follicular dendritic cells (FDC) in the lymph

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nodes as part of the process of immunologic memory. The mAb attaches to the tumor Ag involved in both processes.

MATERIALS AND METHODS

Group 1

Two patients had standard RAID studies elsewhere using the mAb In-111-B72.3; abnormal foci were demonstrated that, on subsequent surgical exploration and tissue examination, proved not to be tumor. Their histories, images, operative and pathological reports, and tissues slides were obtained and reviewed. The tissue blocks, embedded in paraffin, were obtained and recut. Immunostaining was carried out using CC49 (Signet Laboratories, Dedham, MA), a second-generation mAb to a more purified form of the TAG 72 Ag, and 784, a mAb to CD21 (Dako, Carpinteria, CA), a receptor found on FDC, and conterstained with hematoxylin and eosin (H&E). CD 21 is a receptor for the C3d fragment of the third component of complement. The B72.3 mAb was developed to the tumor-associated glycoprotein 72, present in many tumors [10].

Group 2

A similar review of the records and study of the tissues obtained at surgery at this institution in 13 patients undergoing colon resection for primary colon carcinoma was also carried out. These selected consecutive cases, as defined by the American Joint Commission on Cancer (AJCC) TNM staging system [14], had metastases in regional lymph nodes. Patient follow-up studies were not carried out. An additional outside case is included.

RESULTS

Group 1

Case 1. A 52-year-old man had undergone resection of a rectal cancer 7 years previously. On physical examination, he had recurrent tumor in the true pelvis. A CEA test result (<0.5 ng/ml) was within the normal range. A RAID study was obtained to look for tumor spread. It demonstrated multiple foci of increased activity in the retroperitoneal area, suggesting metastatic tumor in lymph nodes (Fig. 1). At surgery the next day, the recurrent tumor was removed from the rectal area. Exploration of the mid-abdomen revealed no tumor; six regional, four para-aortic and two vena caval lymph nodes were examined by standard H&E-stained slides; no tumor was detected.

Our immunohistochemistry (IH) studies of lymph nodes with the CC49 moab revealed only sparse but nontumor cell staining of the GC and Mϕ. Small tumor cell foci were present. Occasional tumor cells were positive for CC49; these cells seem insufficient to cause RAID positivity if the B72.3 mAbs were to demonstrate a similar degree of cell labeling.

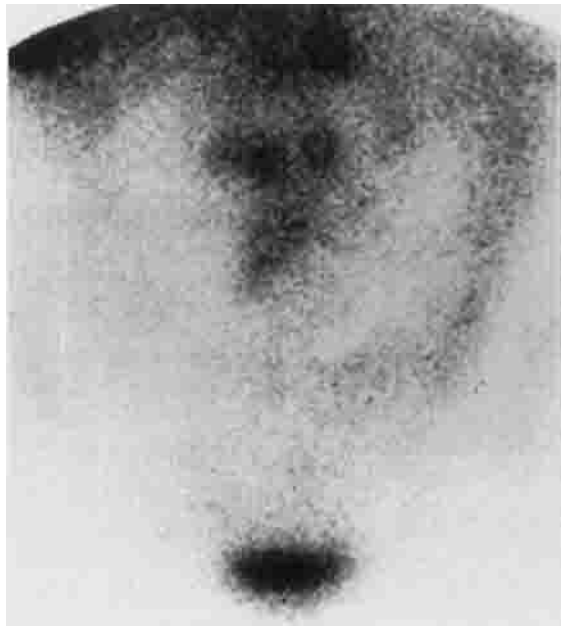


Fig. 1. Case 1. Anterior scintigraphic image 48 hr after intravenous infusion of In-111-DTPA-B72.3 mAb. It demonstrates multiple foci of activity in the retroperitoneal area suggesting metastatic tumor. No tumor was found here at surgery or in the excised lymph nodes.

Case 2. A 68-year-old woman had undergone resection of an ovarian cancer 14 months previously. Tumor extended into the rectum and 3 of 4 pelvic lymph nodes were tumor positive. The entire tumor mass was resected in toto. Serum CA125 3 days postsurgery was normal. Fifteen months later, a RAID study was carried out to evaluate for tumor recurrence. Images at 72 and 96 hr showed multiple focal areas of uptake in the retroperitoneal area, extending down to the presacral region, and also in the right iliac fossa (Fig. 2). Laparotomy 6 weeks after imaging, with thorough inspection, palpation, and multiple biopsies, revealed only two small liver metastases and a small tumor nodule on the left lower pelvic sidewall. Six weeks later, the patient was found at colonoscopy to have recurrent extra-colonic tumor adherent to the coccyx, so it can be presumed that such tumor was present at RAID imaging.

Our IH studies of resected nodal tissue revealed weak but nontumor cell GC positivity, with no Mϕ staining.

Group 2

The pathological and IH features of 14 primary colon carcinomas and the regional lymph nodes (13 were our cases, 1 outside case) are summarized in Table I. All cases were AJCC stage 3 or 4 [14]. Three of three CEA values done within a few days preoperatively were mildly elevated (normal <2.5 for nonsmokers, 5 for smokers). CEA tests were not routine preoperatively, although they were used subsequently for follow-up.

Fig. 2. Case 2. Anterior image of abdomen and pelvis 96 hr after intravenous infusion of In-111-DTPA-B72.3 mAb. Multiple focal areas of uptake in the retroperitoneal area and pelvis. At surgery, no tumor was found in these areas.

We found CC49 positivity in 14 of 14 primary tumors, in 14 of 14 cases in nodal metastatic tumor and, relevant to this report, CC49 positivity of noncellular TAG-72 ag in lymphatic vessels adjoining a colon cancer and in the subcapsular sinus (SS) of a draining lymph node (1 of 14), as well as CC49 staining not due to intact tumor cells in the GC of lymph nodes draining colon cancer (6 of 12), in a pattern similar to that of the stain for FDC (11 of 11) (Fig. 3). In one case, 14 of 22 lymph nodes were GC positive for CC49, including 3 with metastases. Other than this case, CC49 positivity of GC was seen in a minority of lymph nodes and in a minority of GCs within a lymph node. GC formation was strong in the lymph nodes draining colon cancers, occurring in 12 of 14 cases. In two of three cases with elevated CEA, GC positivity was detected. In four cases with annular tumors, GC positivity was present in three; in seven other tumors of known morphology, GC positivity was present in two. In seven cases showing lymphatic invasion by the primary tumor, GC positivity was present in four; in four cases without lymphatic invasion, GC positivity was detected in one (Case 14 excluded). Although the cases are few, there is thus a suggestion that tumor invasiveness is associated with GC positivity.

DISCUSSION

RAID Problem of False Positivity

False positivity was first encountered in RAID studies of 16 colon cancer cases using the In-111-T84.66 mAb to CEA [1]. Focal mediastinal activity in one patient proved to be noncancerous lymph nodes. The In-111 concentration in resected tissue was similar in primary tumors and tumor-positive mesenteric lymph nodes but was more than two times higher in tumor negative mesenteric nodes. The latter could be due to catabolism of large amounts of shed CEA/mAb complexes by Mϕ with sequestration of the In-111 label in their lysosomes (see below). Nondemonstration of these lymph nodes by RAID (with the above single exception) appears due to insufficient total photons. Subsequent RAID reports on a larger number of cases [2–4], in which a second anti-CEA radioindium-labeled mAb, ZCEO25, was also used, resulted in a large proportion of false-positive images in the lymph node region of the abdomen, and thus a low reliability of tumor detection in this area. In a recent report using a third anti-CEA mAb, C110, with an In-111 label, the false-positvity problem was so serious that images positive for abdominal foci outside the liver were considered evidence of tumor only if they were located in "nondraining" lymph node areas [15]. The higher incidence of false positivity, subsequent to the first report [1], may be related to the higher milligram dose of the anti-CEA mAb.

Twelve patients with suspected colon cancer recurrence were studied with radioindium-labeled ZCEO25 and C110 by both RAID and RIGS procedures. False-positive uptake in portal (n = 3), mediastinal (n = 3), and supraclavicular lymph nodes (n = 1) was seen with both methods [6]. False-positive supraclavicular lymph node uptake (n = 3) by both methods was also seen in an ovarian cancer patient using the In-111-labeled B72.3 mAb [7]. Histological sections of suspect areas, with routine H&E stains, were negative for tumor in both studies. The latter study showed sinus histiocytosis, a histologic finding considered to be evidence of immune reactivity to tumor or its products (refs. 3–10 in [16]).

These studies are summarized in Table II. The two Ag involved, CEA and TAG-72, are well known to be shed by tumors [10,11]. False positivity of lymph nodes on RAID studies using mAb to nonshedding Ag has not been reported. Also, the radiolabel has been In-111 only; RAID studies using I-131-B72.3 have not encountered false-positivity problems [17,18].

Our IH demonstration of ag in the lymphatics between tumor and draining node and within the node (group 2) provides evidence for such transport in our two false-positive RAID cases (group 1), from known pelvic tumors to retroperitoneal nodes. Yet in both groups, nodal Mϕ positivity was rare, due to rapid Ag catabolism. In both groups, GC positivity for Ag is evidence for host antitumor reactivity (see discussion below).

RIGS Problem of False Positivity (Table II)

The earliest RIGS report, published in 1989, of false-positive mAb uptake involved the study of breast cancer patients using I-125-B72.3 mAb; 4 of 14 patients had high counts in axillary lymph nodes at surgery 6–26 days post-mAb injection. The lymph nodes histologically dem-

TABLE I. Primary Colon Carcinoma and Draining Lymph Nodes: Pathology and Immunostaining Features

			Tumor									Immunostaining					
						AJCC staging[a]						CD21 (0–4+)		CC49 (0–4+)			
Case	Age/Sex	Preop CEA (ng/ml)	Site	Size (cm)	Morphology	T	N	M	Stage	Grade	Lymph inv (±)	Tumor	Lymph node follicle	Primary tumor	Lymph node tumor	Lymph node follicle	Lymph
1	67 F	ND	AC	6 · 7 · 3	Obstructing, annular	3	2	×	3	2	—	0	2	3	3	0	3
2	30 M	3.5	AC	7 (diam)	Annular	4	2	×	3	3	+	0	2	3	3	1	0
3	83 F	ND	Cecum	4 (diam)	Fungating	3	1	×	3	2	−	0	3	1	1	1	0
4	77 F	ND	Cecum	2 (diam)	Polypoid	3	1	×	3	2	+	0	1	1	1	0	0
5	61 M	ND	DC	2 (length)	Obstructing; annular	3	2	×	3	3	+	0	3	3	2	3	0
6	71 M	ND	AC	2.5 (length)	Fungating; annular, ulcerated	3	1	×	3	2	+	0	3	3	3	2	0
7	87 F	ND	AC	7 · 5	Fungating	3	1	×	3	2	−	0	3	3	3	0	0
8	52 F	ND	RS	5 · 5	Fungating, ulcerated	3	2	1	4	2	−	0	NA	3	3	NA	0
9	88 M	ND	DC	2 (diam)	Polypoid	3	1	×	3	2	−	0	2	1	1	0	0
10	68 M	10.0	Sigmoid	4 (diam)	Fungating	3	2	×	3	2	+	0	2	3	3	2	0
11	75 F	6.5	AC	3.2 · 2.2 · 1.1	Nearly annular, ulcerated	3	1	×	3	2	+	0	3	3	3	0	0
12	61 F	ND	Cecal	5 · 5 · 4	Fungating	3	2	1	4	3	+	0	3	2	1	0	0
13	81 F	ND	Sigmoid	5 (length)	Fungating, annular	3	2	×	3	3	+	0	NA	3	3	NA	0
14[b]	54 F	ND	RS			3	2	1	3	2	−			1	1	2	0

[a]AJCC TNM System [14]: stage 0 = Tis N0 M0; I T1 N0 M0; T2 N0 M0; II = T3, 4 N0 M0; III = T1–4, N1–3 M0; IV = T1–4 N1–3 M1; definition of TNM categories: T, depth of primary tumor invasion: Tis, carcinoma in situ, T1 = mucosa or submucosa, T2 = muscularis propria, T3 = subserosa or periocolic tissue, T4 = through serosa or invading contiguous organs; N, metastasis in regional lymph nodes: N0 = no positive nodes, N1 = 1–3 positive nodes, N2 = 4 or more positive nodes, N3 = any positive node along a named vessel; M, metastasis in distant organs: M0 = absent, M1 = present, MX = minimum requirements for assessment cannot be met.

[b]Outside case.

Lymph inv, lymphatic invasion by primary tumor.

AC, ascending colon; DC, descending colon; RS, rectosigmoid N0 M0, T2 N0 M0.

ND, not done; NA, not applicable (no follicles demonstrated).

onstrated only sinus histiocytosis [5]. Two additional reports of RIGS utilization, noted above [6,7], described high but false-positive count rates in lymph nodes suspected of harboring metastatic cancer. RIGS studies of 36 patients with primary or suspected recurrent colorectal cancer were carried out 2–3 weeks after injection of the I-125-CC49 mAb [8,9]. This mAb is directed against a different combining site on the TAG 72 molecule than B72.3 [10], but the same host catabolic and immunologic mechanisms would apply. Most interesting were those lymph nodes, classified as type III, that is, RIGS positive, histology (H&E) negative for tumor. Elevated γ-ray count ratios, compared to normal tissue, were similar to those of type IV tissues (RIGS positive, histology positive for tumor). A retrospective study of 15 type III lymph nodes revealed that 6 of 15 were in fact positive for micrometastases, as shown by repeat H&E stains of serial sections or cytokeratin (ck) staining using 2 anti-ck mAbs specific for epithelial cell keratins (antikeratin AE1/AE3 [19]). Cytokeratin staining occurred in carcinoma cells. Four of 15 lymph nodes, however, were positive with CC49 immunostaining, in nontumor cell elements. The remaining four nodes were not discussed but were presumably RIGS positive, tumor cell negative, and CC49 negative; these lymph nodes raise a question of the relative sensitivity of RIGS and IH. Unlike RAID, RIGS false positivity was demonstrated using radioiodinated mAbs.

THE SEARCH FOR THE CAUSE OF RAID/RIGS FALSE POSITIVITY: RADIOCHEMISTRY

Indium-111

In a study of colon cancer patients who had received In-111-labeled anti-CEA mAb for RAID studies 5–20 days before surgery, the radioindium concentration was similar in tumor-positive and tumor-negative draining mesenteric lymph nodes, but the CEA concentration was 10 times lower in the latter [3]. It was recently shown that In-111-DTPA-labeled glycoproteins (models of Abs), once internalized in a cell, are catabolized, with In-111-DTPA-polypeptides delivered to the lysosomes. Here further degradation occurs, to In-111-DTPA-lysine, which remains in the lysosome because it can neither diffuse across the lysosomal membrane nor use existing transport systems [20]. Thus, in the lymph nodes of false-positive RAID/RIGS cases, In-111 persists in the lysosomes of the Mϕ or B cells, following catabolism and Ag processing in these cells of the CEA or TAG-72 or other tumor Ag, and of the In-111 mAb attached to the tumor Ag. Such lymph nodes could now be Mϕ negative for tumor Ag on IH, as in our cases 1 and 2.

Radioiodine

The metabolism of (radio)iodine is markedly different from that of (radio)indium. Many antitumor mAbs, following attachment to tumor cells, are internalized and degraded, chiefly in the lysosomes [21]. If labeled with radioiodine at the tyrosine residue, as in I-125-B72.3 [22], iodotyrosine would be rapidly released from the cell [21] and excreted in the urine. However, I-125-B72.3 has been shown to be retained in colon tumor grafts in nude mice for at least 21 days postinoculation [22], probably due to noninternalization. This mechanism could explain RIGS detection of colon cancer foci in humans for several weeks after injection of I-125-CC49, an Ab to the same TAG-72 Ag [8,9]. But for tumor-negative lymph nodes, RIGS positivity is still evidence for an extracellular location of the radioiodine, which could be explained by attachment of the I-125-CC49 to the Ag portion of the Ag/Ab complexes affixed to the FDC (see discussion below). Evidence for the long-term persistence and integrity of Ag/Ab complexes in lymph nodes is provided by the observation that mice, previously immunized to human serum albumin (HSA), when injected in the footpad with the same Ag labeled with radioiodine, retained the Ag in undegraded form and still radiolabeled, in the follicles of the popliteal lymph node 12 weeks later. In nonimmunized mice, the same I-125-HSA disappeared rapidly, the difference already evident by day 6 [23].

THE SEARCH FOR THE CAUSE OF RAID/RIGS FALSE POSITIVITY: IMMUNOHISTOCHEMISTRY

The group that carried out the above RIGS/IH comparisons [8,9] described an IH study of 568 pericolic (i.e., draining) lymph nodes in 50 patients with colorectal cancer, stage Dukes B (transmurally invasive with negative lymph nodes on H&E-stained slides) [19]. Staining with the CC49 mAb was positive in 304 lymph nodes (54%) from 38 patients. This staining showed two distinct patterns, in the follicles, and in sinus histiocytes (Mϕ). Together or alone, neither pattern correlated with patient survival over a 7-year follow-up period. Thus such CC49 localization could not be attributed, overall, to intact cancer cells. They did not give the proportion attributable to each pattern, nor did they describe the pattern of follicular positivity.

Processing of Exogenous Antigen in the Lymph Nodes

It is the premise of this report that noncellular tumor components are processed in the draining lymph nodes in the same manner as is exogenous Ag. Such components are recognized as nonself by the immune system, and an immune response is generated.

Antigen in transit. After injection of radiolabeled soluble Ag (I-125-HSA) into the subcutaneous tissues of the lower leg of sheep, it was detected in the efferent lymph of the primary lymph node, the popliteal, within minutes. By 24 hr, 75% of the dose was collected. In immune sheep, efflux from the primary node was only

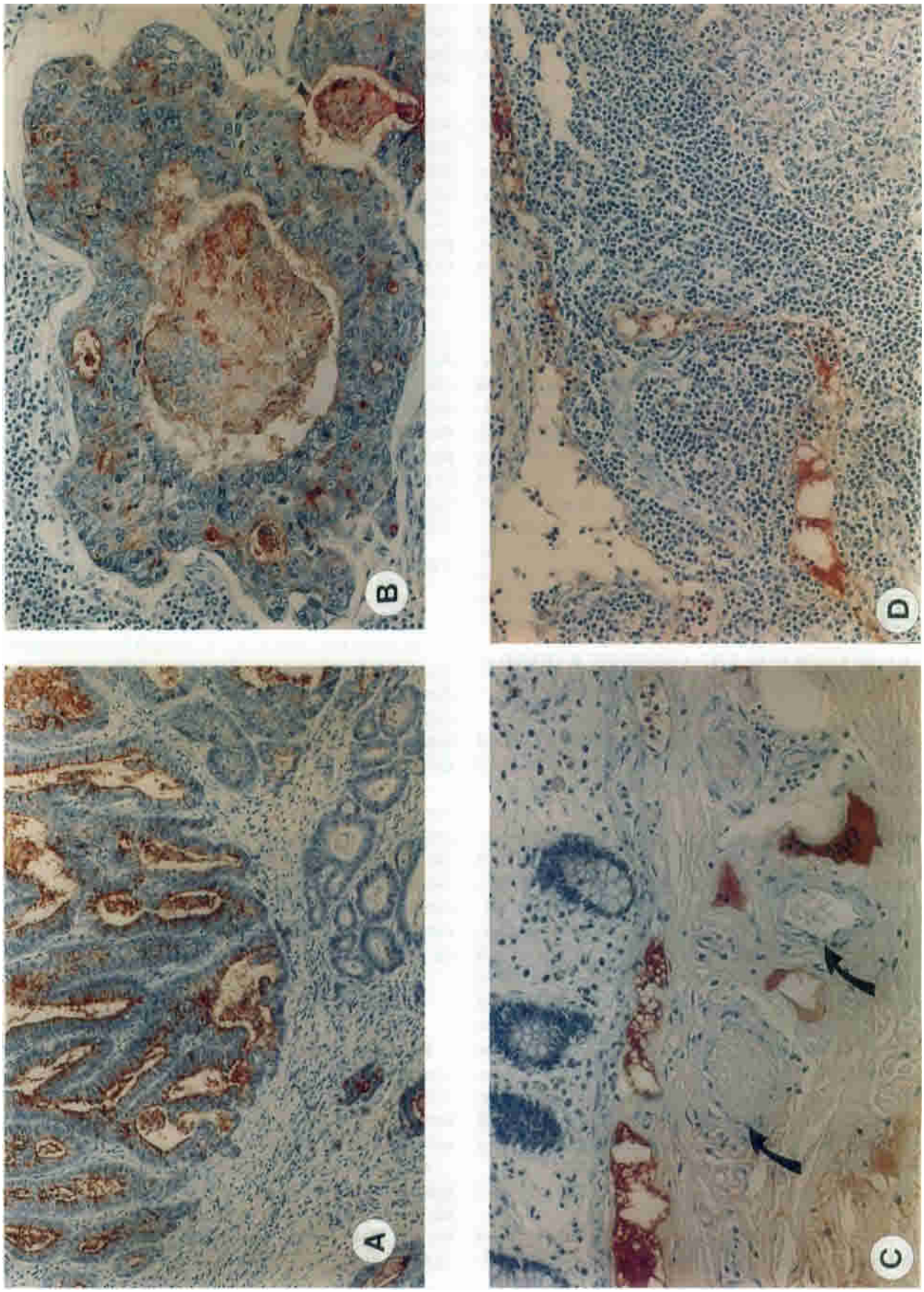
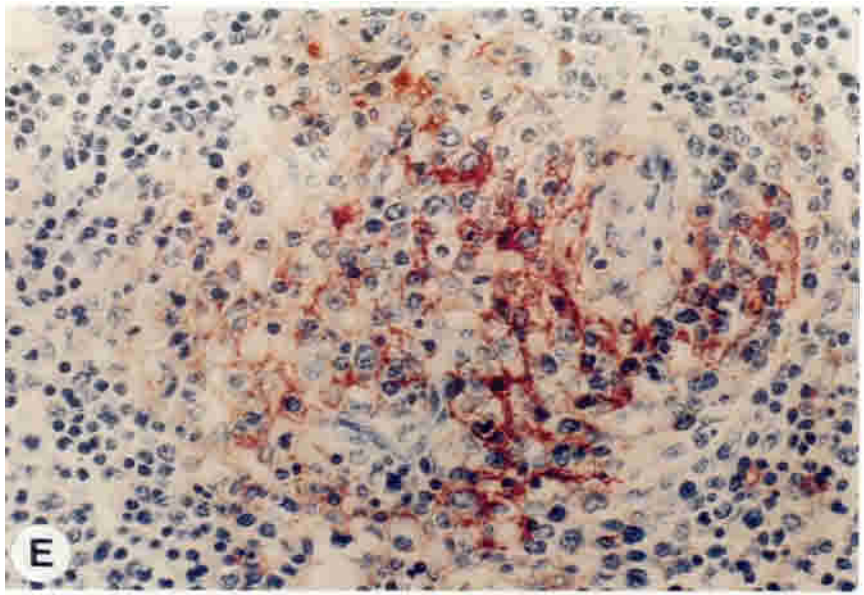
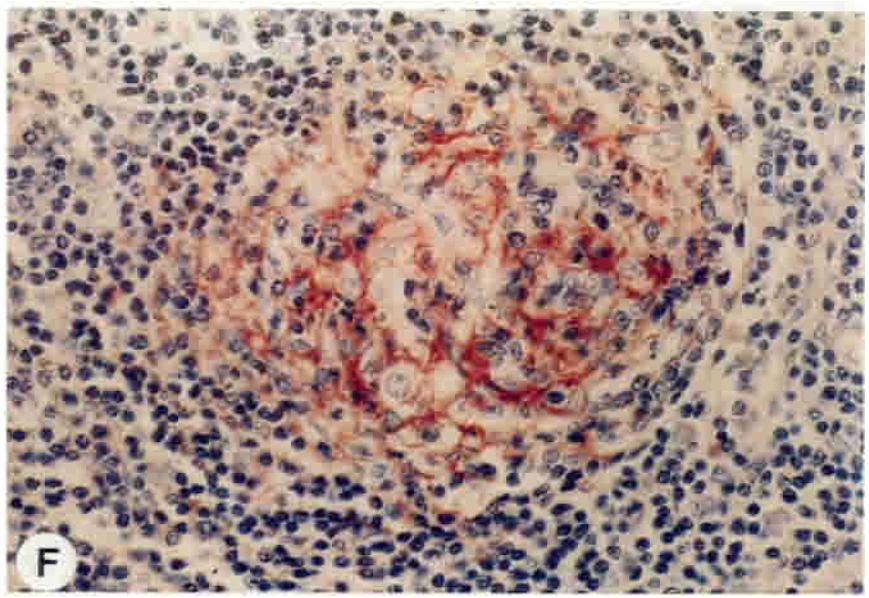


Fig. 3.





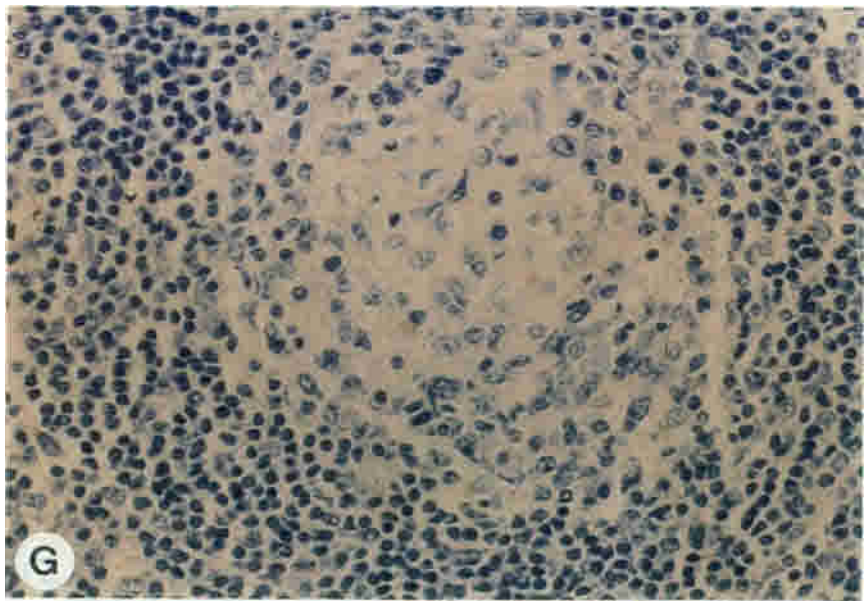


Fig. 3 (continued). Immunohistochemical studies of tissues from patients with colon cancer. Counterstain: hematoxylin and eosin. **A–E:** Distribution of tumor antigen recognized by CC-49 monoclonal antibody. **A:** In primary colonic adenocarcinoma. X100. **B:** In metastasis in lymph node. X200. **C:** In lymph, in lymphatic channels in the colonic submucosa (note absence of staining in blood vessels, an arrowed). X200. **D:** In lymph, in sinuses in a benign mesenteric lymph node draining the region of the primary tumor. X200. **E:** On membranes of cells in germinal centers of a benign reactive lymph node. X400. **F:** Distribution of follicular dendritic cells in the germinal center of a benign reactive lymph node, as shown by staining with an antibody to CD-21 (note similarity to E). X400. **G:** Negative control section similar to that illustrated in E and F, stained using normal mouse serum in place of primary mouse monoclonal antibodies. X400.

TABLE II. RAID and RIGS Problems of False Positivity: Published Studies; Surgically Proven

Antigen	Label/mAb	Dose (mg/mCi)	Tumor	Incidence (pts/pts or foci(f)/pts)	Site[a]	Reference
RAID						
1. CEA						
	a. In-111-T84.66	0.2/2	Colorectal	1/16	2	1
	b. In-111-ZCE025	20–40/2.5–7	Colorectal	7/45	1	2–4
				2/45	2	2–4
	c. In-111-ZCE025	40/4–6.5	Colorectal	3f/12	1	6
	In-111-C110	5/4–6.5	Colorectal	4f/12	2	6
	d. In-111-C110	5/5	Colorectal	6/51	1	15
2. TAG-72						
	a. In-111-B72.3	1/5	Ovarian	1/5(3f)	2	7
			Colorectal	0/8		7
	b. I-131-B72.3	0.2–2/0.8–10	Colorectal	0/35		17
RIGS						
1. CEA						
	a. In-111-ZCE025	40/4–6.5	Colorectal	3f/12	1	6
	b. In-111-C110	5/4–6.5	Colorectal	4f/12	2	6
2. TAG-72						
	a. I-125-B72.3	1/5	Breast	4/14	2	5
	b. In-111-B72.3	1/5	Ovarian	1/5(3f)	2	7
			Colorectal	0/8		7
	c. I-125-CC49	0.2–10/1–2	Colorectal	?[b]/17	1	8,9

[a]Site 1, intra-abdominal extrahepatic; site 2, extra-abdominal.
[b]Not stated; 4 of 15 lymph nodes were RIGS positive, CC49 positive on IH, tumor cell negative.
RAID, radioimmunodiagnosis; RIGS, radioimmunoguided surgery; CEA, carcinoembryonic antigen; IH, immunohistochemistry.

slightly less (69%) [24]. The radioactivity in the efferent lymph was not associated with cells, i.e., Mϕ.

Classic (Macrophage) Pathway of Antigen Processing (Catabolism)

Antigen processing in the lymph node involves two basic processes: pure catabolism and immunological processing. Immunological processing differs for primary and secondary exposure to Ag. The primary immune response is probably not relevant in explaining false-positive RAID/RIGS studies of cancer patients because the immune system of the host has long been exposed to putative tumor Ag and reacted to them.

In previously immunized rabbits, as in naive rabbits, subcutaneously injected soluble Ag localized, in the primary draining lymph node, mainly in medullary sinus Mϕ [25]. It has been shown in passively immunized mice that subcutaneously injected immunogen (e.g. the histochemically identifiable Ag, horseradish perosidase) is already complexed with Ab (in the afferent lymphatics), when it enters the subcapsular sinus (SS) of the lymph node [26]. These immune complexes because of their larger size do not percolate freely through cortex, but are more restricted to the SS of the lymph node than is soluble Ag. Most of these complexes are found in sinusoidal Mϕ [26,27], chiefly in the efferent side of the SS, and in the medullary sinuses. These Mϕ are not involved in the induction of secondary immune responses [26]; they are purely catabolic. This is the classic (i.e., Mϕ) pathway of Ag processing. Quantitatively, this is by far the larger component of Ag disposal following injection into immune animals (Fig. 4).

Alternative (Nonmacrophage) Pathway of Antigen Processing

In the rabbit experiments cited above [25], which were carried out over a period of 6 hr to 21 days after Ag injection, in addition to Ag uptake by medullary Mϕ, germinal center localization of Ag was observed throughout the period in immune animals, but in normal animals only after Ab was detectable in the serum. The immune complexes attach to the FDC, located in the GC, by means of receptors for the Fc and C3 components of the complexes [28]. The GC thus becomes an Ag-retaining reticulum, a three-dimension network formed by the interdigitating Ag-retaining dendritic processes of the FDC, a reservoir of retained Ag in the form of Ag/Ab complexes [29]. During days 1–3, dendrites of some of the FDC become beaded [26]. These beads break off, forming immune complex-coated bodies (iccosomes). At about day 3, the GC enlarge by becoming edematous, facilitating the dispersal of iccosomes [26]. By means of their immune complex coat, iccosomes attach to, and are then endocytosed by, B cells of the GC, their Ag undergoing processing within the B cells, followed by Ag presentation by these B cells to helper T cells (Th). The cells in turn

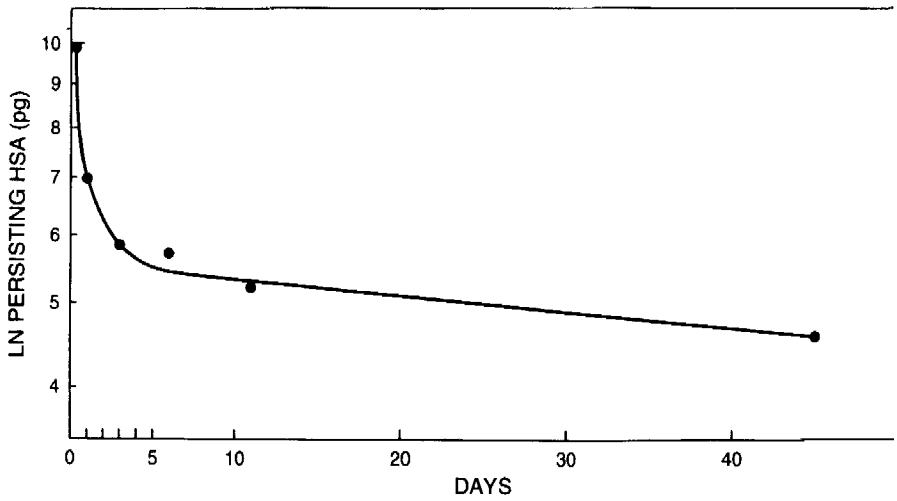


Fig. 4. Clearance of I-125-HSA from the popliteal lymph nodes of immunized mice after bilateral hind footpad injection. The dose was 2.5 μg at each site. Semilogarithmic plot of data from Figures 1 and 2 [31]. Reprinted from Tew et al. [31] with the permission of the authors and Publisher (Blackwell Scientific Publications).

stimulate Ab production by specific B cells. The latter migrate to the medullary cords and become plasma cells, which may then disseminate. Antibody production peaks at 3–5 days, then declines. At about day 10, memory B cells are formed [26]. Thus, exposure to Ag results in a two-armed humoral response: Ab production and immunological memory.

Antigen Persistence in Lymph Nodes: Immunological Memory

In a landmark experiment [30], mice were given a unilateral hind footpad injection of horseradish peroxidase (a soluble Ag) with a booster dose 2 weeks later. At 2 weeks and 2 months after the booster Ag injection, high counts of antibody-forming cells (AFC) were found in the ipsilateral popliteal lymph nodes, with AFC, although fewer, in the more centripetal lymph nodes, such as the para-aortic nodes, and even in the spleen. One year later (the longest interval studied), AFC counts had disappeared or declined in all lymph nodes, but were still highest in the ipsilatereal popliteal lymph node [30]. Removing the popliteal lymph nodes from mice injected with Ag in the hind footpads resulted in a marked decrease in serum Ab titer [29]. I-125-HSA injected into the hind footpads of immunized mice was recovered from the draining popliteal lymph node 12 weeks later, in intact form; it was localized to the follicles [23]. Electron microscopy of FDC has provided confirmatory evidence for Ag persistence. By day 14 after exposure to Ag, some FDC show a tightening of the convolutions of their filiform dendrites, thus reducing the exposure of the retained immune complexes to surrounding B cells [26]. These and other studies support the concept that Ag persisting on FDC is responsible for the maintence of the systemic humoral immune response [29]. Whenever Ab levels decline, FDC present Ag to memory B cells and a new cycle of Ab synthesis is initiated; this newly formed Ab raises the Ab titer, terminating the immunologic stimulus [31].

Tumor Immunology

Studies of spontaneous human tumors have demonstrated convincingly that many tumors express Ag, which can induce both cellular and humoral immune responses in the host [32]. The successful development of human antitumor mAb using B cells from the draining lymph nodes of carcinoma patients indicates that spontaneous human tumors naturally elicit host antitumor immune responses in vivo [33,34]. The mAb cited in this report, to CEA and TAG72, were produced by hybridomas that are a fusion product of murine spleen and myeloma cells [35]. While it could be questioned whether the immune reactivity of such murine cells to a component of a human tumor should predict immune reactivity of the human tumor host to the same component, a highly experienced authority has noted that Ag of human tumors recognized by mice and by humans are often the same; an example is the p97 Ag of melanoma [32]. Furthermore, the epitopes could differ. Since such parallel reactivity has not been observed frequently [36], it follows that each tumor Ag recognized by murine hybridoma cells and for which a mAb has been developed, may be a separate problem in regard to demonstration of human host reactivity to the same Ag and, by extension, if the Ag is shed, of false-positive RAID/RIGS studies due to follicular deposition of Ag/Ab complexes.

Recent thorough reviews conclude that circulating immune complexes are present in most cancer patients, in-

clude those with bowel cancer [37,38]. The incidence and level of complexes increase with the tumor burden. The complexes may be small and noncomplement binding, which could explain the low incidence of overt renal and other systemic complex-related diseases in cancer patients [38], and by extension perhaps also the variable incidence of follicular localization in the draining lymph nodes as described in this report. Immune deposits have been detected by electron microscopy in the kidneys of one-half of a group of cancer patients with otherwise undetectable renal disease [39].

Patients with colon cancer have frequently demonstrated CEA/Ab complexes in their blood (refs. in [37,38]), which is evidence of immune reactivity to CEA. In a recent paper, high titer Ab to CEA was reported in 14 of 25 (56%) patients with colon cancer, with a higher percentage in patients with no metastases [40].

With the TAG-72 Ag, questions arise. Neither immune complexes nor free Ab in the serum has been reported involving the TAG-72 Ag in colon cancer patients, but the IH findings by our group and others are strongly suggestive of anti-TAG-72 immunization. We have shown GC positivity to CC49, in tumor cell-negative GC, indicating the presence of TAG-72 Ag. The pattern was similar to the GC distribution pattern of FDC. FDC are well known as sites of deposition of Ag/Ab complexes, which attach to Fc and C3 receptors for IgG and C components of the complexes. The genesis of immune complexes in naive hosts may be instructive. It has been shown that 6 days following primary intravenous immunization of rabbits with two different soluble Ags, separate rounded discrete foci of GC localization of the resulting two different ag/ab complexes were seen in the spleen. This is a situation of Ag excess. Since FDC are nonspecific in their trapping of immune complexes, such a pattern indicates local formation of immune complexes, from Ab released by local Ab-forming cells combining with Ag present in excess between the cells, followed by trapping of the complexes on closeby FDC [41]. If host tumor Ag such TAG-72 is present in the serum (Ag excess), as has been observed in patients with certain tumors [10], free host Ab to it or Ag/Ab complexes might not be detectable in the serum, despite GC deposition, and especially if the host immune response were weak. It has been noted that in in vitro spleen cell cultures, Ag/Ab complexes can exert Ab feedback suppression of the Ab response: this suppression was determinant specific, suggesting that it was due to blocking of epitopes ([42] and references cited therein). Since GC formation is strong in the lymph nodes draining colon cancers, suggesting strong B memory cell generation, while serum ab to TAG-72 has not been detected, the final answer may well depend on the relative strengths of the two arms of the GC response: the humoral response versus memory B cell formation [43].

Four other less likely possibilities of false-positive lymph node images can be noted: cross-reactivity of both of the radiolabeled anti-TAG-72 mAbs (B72.3, CC49) and the anti-CEA mAbs with lymph node Ag; processing of such mAbs themselves as Ag; formation of mouse–antimouse Ag/Ab complexes and antiidiotype Abs. The first would have been detected during the early thorough IH screening of these mAbs [10,44] and would involve lymph nodes anywhere in the body. With regard to the second and third, intravenously presented Ag is processed mainly in the spleen [45] and Mϕ catabolism of Ag or complexes and deposition of complexes on FDC would be similarly localized. Following multidose I-131-CC49 treatment of patients with metastatic colon cancer, all patients developed antimouse Ab to this Ag and, with the second treatment, body clearance occurred more rapidly. The spleen was seen on scintigrams, consistent with uptake and digeston of CC49/anti-CC49 complexes [46]. Patients undergoing diagnostic RAID/RIGS studies have received only a single dose of murine mAb; human antimouse Ab (HAMA) formation in these protocols has occurred in a minority of such patients [47,48]; also HAMA formation would occur only after some days, as in a primary immune reaction, so it would not be a factor in RAID studies. Also, such explanations would not apply to IH studies of lymph nodes from cancer patients who did not receive the murine mAb in vivo. Finally, antibody/anti-idiotypic antibody complexes, if formed, would be trapped and retained by the FDC, as are putative original Ag/Ab complexes [49].

Comparison of the Classic and Alternative Pathways of Antigen Processing

Figure 4 illustrates the quantitation and kinetics of the processing of soluble Ag by primary lymph nodes in immunized mice [50]. The curve is biphasic. Following footpad injection, the draining popliteal lymph node at 4 hr contains 0.8% of the injected dose. At 24 hr, it has dropped to 1/19 and at 3 days to 1/57 of the 4-hr value respectively. This rapid drop is followed by a slow decline thereafter. The study ended at 45 days. The half-life of the plateau portion of the curve was 8.1 weeks [50]. These mice had been injected in both hind feet. On day 11, unilateral footpad amputation was carried out; Ag level in the ipsilateral popliteal lymph node remained similar to that of the contralateral popliteal lymph node when measured 8 and 35 days later. This indicated that the plateau shown in Figure 4 was not due to slow flow of I-125-Ag from the footpad injection depot but was due to intranodal persistence. Earlier experiments by these workers [50] provided evidence that nodally retained radiolabel represented retained and undegraded Ag.

Autoradiography of the lymph node showed that the early almost vertical decline in the curve was due to the rapid uptake and catabolism of most of the Ag in Mϕ of the SS and medullary sinuses (classic pathway of Ag

processing) [27, 50]. (In vitro experiments by the same workers on murine peritoneal Mϕ revealed a half-life of ingested I-125-HSA/anti-HSA complexes of 2 hr [50].) A small amount of Ag was also present, at this time, in the superficial cortex and follicles. By 24 hr most of the Ag had been cleared from the Mϕ, but follicular uptake was stronger. By day 6, retained Ag was present only in the follicles [50], that is, in the alternative (non-Mϕ) pathway.

Relevance of Antigen-Processing Pathways to False RAID/RIGS Studies

The tumor Ags detected by moabs in this report, CEA and TAG-72, are known to be shed by tumors. CEA is frequently elevated in the blood of patients with colon cancer [51], and its level reflects tumor spread [52]. False IH (i.e., non-tumor cell) positivity in lymph nodes has also been noted more frequently in patients with high blood CEA levels and large tumors [6], indicating the importance of ag excess. Blood TAG-72 Ag level is also elevated in various cancers [10], including colon cancer; similarly, the level may reflect tumor mass [53]. Our studies were retrospective and were directed at etiologic mechanisms, but in three cases in group 2 with elevated CEA, two had false GC (nontumor cell) positivity for TAG-72. Patients could have a normal blood Ag level yet false-positive RAID or RIGS, if tumor Ag does not escape the draining lymph nodes, into the blood, as may be the situation in case 1, group 1.

If shed tumor components are handled in lymph nodes similar to soluble exogenous Ag, concepts applicable to the latter would apply, that is, the rate of shedding, the rate of flow in the draining lymphatics, and their ultimate metabolic fate will determine their RAID/RIGS demonstration. First, and most simply, we have demonstrated noncellular TAG-72 ag by IH in transit in the subcapsular sinus of a lymph node draining a colon cancer. Such Ag should be accessible to mAb, such as In-111-B72.3, given intravenously, and contribute to scintigraphic positivity of primary or even secondary lymph nodes [24]. Second, uptake of Ag or immune complexes would occur in the Mϕ of draining lymph nodes. IH studies have shown CEA within histiocytes (Mϕ) of lymph nodes draining colon cancers [6]. Large liver metastases can produce a high noncellular Ag flow to the portal hilar lymph nodes, explaining the RAID/RIGS positivity, which has been shown surgically to be benign (i.e., non-tumor cell) and which has been associated with nodal Mϕ positivity on IH for TAG-72 Ag [6]. In fact, based on experience, surgeons operating on these patients dismiss such portal hilar radiopositivity as not due to metastatic tumor cells but rather to noncellular Ag [6]. Because of the rapidity of catabolism in Mϕ [31], RAID/RIGS and IH positivity due to Ag in Mϕ represents current Ag flow. In addition, using In-111-labeled mAb, RAID/RIGS positivity is clearly possible, despite Mϕ negativity for tumor Ag by IH, because of lysosomal retention of the radiolabel, the explanation for our cases. A third process, follicular deposition of Ag, could be due to current Ag flow with Ag demonstrated, as part of Ag/Ab complexes, on antigen transport cells [27] or FDC or during B-cell processing. In RIGS, the persistence for weeks of an I-125 label is evidence of an extracellular localization of the mAb. We have demonstrated follicular uptake of CC49 mAb by IH in lymph nodes draining colon cancer, in a pattern similar to the location of FDC, providing evidence for the presence of Ag, with the implication of Ag/Ab complexes attached to FDC. In the presence of sustained large Ag flow to lymph nodes from a tumor, all these processes, that is, Ag in transit, the Mϕ and non-Mϕ pathways could be expected to be IH positive and to contribute to RAID or RIGS false positivity. Cessation of Ag flow would rapidly eliminate the first cause. A correlation of nodal RIGS positivity and CC49 staining of their GC with no Mϕ positivity has been reported [8,9]. In view of the small amount of putative tumor ag deposited as immune complexes on the FDC of the GC, compared to the catabolic capacity of the Mϕ (Fig. 4), such deposition seems unlikely as a cause of RAID positivity but could cause RIGs positivity, since the latter is far more photon sensitive. In RIGS, the radiation detector is very close to the tumor. Since the duration of persistence of putative ag/ab complexes of tumor origin on FDC in lymph node follicles, as an immunological memory phenomenon, is unknown, the possible duration of false positive RIGS and IH studies in the lymph nodes draining a tumor due to them, such as after tumor removal, is unknown. The duration of false positivity has not been addressed as a valid clinical concern and has not been examined in published IH studies of lymph nodes from the drainage area of colon cancers. This is not a theoretical question, since such false positivity after colon cancer removal might be considered, incorrectly, as evidence of ineffectual surgery.

Further studies by us involve more detailed elucidation of the mechanisms of tumor Ag retention in the follicles of the lymph nodes draining colon cancer. The lymph nodes draining other cancers will then be examined to explore the generality of this phenomenon. This area of tumor immunology needs further study.

Our cases were advanced cases. Knowledge of possible false RAID/RIGS positivity may be more important in earlier stages of colon cancer, in which surgical cure is more likely. In regard to avoidance of false positivity, RAID and RIGS studies with an In-III label on the antitumor mAb should be avoided. For RIGS studies using the I-125 label, awareness of the possibility and thorough frozen section evaluation of excised tissues during surgery are indicated.

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